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(54) Title of the Invention: A Beautifying and Whitening Cosmetic Material

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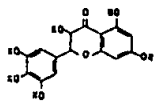
Specification

1. Title of the Invention

A Beautifying and Whitening Cosmetic Material

2. Claims

(1) A beautifying and whitening cosmetic material in which dihydromyricetin as represented by the general formula shown below is the effective component.



(2) A beautifying and whitening cosmetic material as described in Claim 1 in which the dihydromyricetin content is 0.001-5.0 weight %.

3. Detailed Description of the Invention

[Field of Industrial Use]

This invention relates to a novel skin beautifying and whitening cosmetic material. In greater detail, it relates to a whitening cosmetic material with a great beautifying and whitening effect that contains dihydromyricetin ($C_{15}H_{12}O_9$; Ampelopsin; which is also referred to as 3,5,7,3',4',5'-hexahydroxyflavanone and is hereafter referred to as dihydromyricetin) as its effective component.

[Prior Art]

There are many unclear points about the mechanism of occurrence of skin blotches, dark skin and freckles. However, it is generally thought that the causes are hormone abnormalities and stimulation by ultraviolet rays from sunlight, with the pigment melanin being formed and being abnormally deposited in the skin. The methods of treatment of blotches and freckles of this kind include methods in which a substance that inhibits production of melanin, for example, L-ascorbic acid, is administered orally in a large dose, methods in which glutathione is injected and methods in which L-ascorbic acid or cysteine are applied locally in the form of an ointment, cream or lotion. In Europe and the United States, hydroquinone preparations are used as treatment drugs for blotches and freckles.

[Problems the Invention is Intended to Solve]

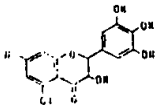
L-ascorbic acids present a problem of stability over time and are particularly unstable in systems containing water and are causes of discoloration and changes of odor. On the other hand, although hydroquinone is highly effective, sensitization to it occurs, for which reason its use is limited. In addition, it is readily

oxidized in air, for which reason stability is a problem. Thiol compounds such as glutathione and cysteine have very strong unpleasant odors, are readily subject to oxidation and their effects are gradual. In addition, it is known that 2-mercaptoethylamyl hydrochloride and N-(2-mercaptoethyl)dimethylamine hydrochloride decolorize black guinea pigs. However, these compounds are not only unstable but are also strongly irritating, with white spots tending to develop after discoloration. Therefore, they are not generally used.

[Means for Solving the Problems]

In the light of this information, the inventors conducted intensive and repeated research, and, as a result, arrived at this invention by finding that the flavonoid dihydromyricetin exhibits an excellent skin beautifying and whitening effect.

Specifically, this invention is a beautifying and whitening cosmetic material in which dihydromyricetin as represented by the general formula shown below is the effective component.



The dihydromyricetin of this invention may be a synthetic product or it may also be a substance that is extracted from natural substances. When it is a natural product, it does not have to be a pure product but may be a mixture that contains dihydromyricetin.

A method that can be used for its extraction from natural substances can include efficient fractionation and extraction methods such as, for example, those described below. Following the method of Mizuno et al, (Tamao Mizuno, Toshiyuki Tanaka, Munekazu Iinuma, Yuko Kimura, Hiroyoahi Ohashi and Hideki Sakai[?], Collected Abstracts of the 32nd Annual Meeting of the Japanese Natural Drugs Society, p. 51, 1985, Okayama), the mature leaves of the plants of the family Salicaceae, Salix sachalinensis Dr. Schm. and Chosenia bracteosa Nakai, are extracted with methanol and concentrated, after which they are distributed using ether followed by ethyl acetate. The ethyl acetate layer that is obtained is fractionated and purified by silica gel column chromatography and the drugs are obtained. In execution of this invention, the quantity of dihydromyricetin that is compounded should be 0.001 to 5 weight %, and, preferably, 0.005 to 3 weight %, of the total quantity of the cosmetic material. When it is less than 0.001 weight %, the effect of this invention cannot be sufficiently manifested. This is not desirable. Although compounding of greater than 5 weight % is possible, no marked intensification of the effect is seen and it is not advantageous economically.

Various components that are generally used in cosmetic products, i.e., oils and fats, waxes, hydrocarbons, fatty acids, alcohols, synthetic esters, surfactants, humectants, thickeners, inorganic substances, perfumes, drug preparations and water can be compounded in addition to the aforementioned essential components of the cosmetic material of this invention within a range that does not impair the effect of the invention.

[Effect of the Invention]

The beautifying and whitening cosmetic material of this invention has a strong inhibiting effect on tyrosinase. As disclosed in a patent application (Japanese Patent Application 62-041173 [1987] by the applicants, it is of high stability and is soluble in water and alcohols. Therefore, it is readily useable as a cosmetic material.

Next, we shall describe the effect of this invention by presenting examples.

[Example 1]

Dihydromyricetin was dissolved in water and an aqueous solution of a concentration of 1.0 mM dihydromyricetin was obtained. The results of a study of the tyrosinase activity inhibiting power of this aqueous solution are described below.

1 ml of L-tyrosinase solution (0.3 mg/ml), 1 ml of Macklebane's [phonetic]* buffer solution (pH 6.8) and 0.9 ml of aqueous solution of dihydromyricetin were added to a test tube and the mixture was incubated for 10 minutes in a constant temperature water tank at 37°C, after which 0.1 ml of tyrosinase solution (1 mg/ml) was added and the mixture was stirred. Absorbance at 475 nm was then at once determined over time with a spectrophotometer.

In addition, as a blank test, absorbance was determined in the same way using water instead of the aforementioned aqueous solution.

Next, as a comparative example, an aqueous solution was prepared in the same way as in the example using ascorbic acid and a study was conducted of its tyrosinase activity inhibiting power.

The results of the various tests in these examples and comparative examples are shown in the appended figure (graph). From this graph, it can be seen that the aqueous solution obtained in Example 1 had a more marked tyrosinase activity inhibiting power than the aqueous solution comprised of ascorbic acid. Further, the fact that the aqueous solution comprised of dihydromyricetin was stable over time and that it had a fixed inhibiting power by comparison to the tyrosinase activity inhibiting power of ascorbic acid, which decreased over time, is characteristic of this invention.

Next, we shall illustrate the beautifying and whitening effect of cosmetic material of this invention by means of Example 2.

* Transliterated phonetically from the Japanese. As such, the spelling may differ from other transliterations.

[Example 2]

The quantities of dihydromyricetin compounded were varied and its beautifying and whitening effect was studied. The quantity of dihydromyricetin compounded in the toliet water of Example 1 was varied and the test materials (Nos. 1 to 8) shown in Table 1 were prepared.

Table 1

Test Material No.	Quantity of dihydromyricetin compounded (weight %)
1	0
2	0.001
3	0.005
4	0.01
5	0.1
6	1.0
7	3.0
8	5.0

A panel of 40 individuals complaining of dark skin, blotches and freckles was constituted and tests were conducted with each test material being assigned to 5 individuals. The toilet water was applied to the face each day for one month and evaluations were made of its effect in rendering color lighter on the basis of the following evaluative criteria. The results of the evaluations are shown in Table 2.

< Evaluative Criteria >

3 points: Pigment deposition was not pronounced [NOTE: More literally, lost its prominence]

2 points: There was very faint pigment deposition.

1 point: There was some pigment deposition.

0 points: There was no change in pigment deposition.

Table 2

Test Material No.	Total scores of 5 test subjects
1	1
2	8
3	12
4	14
5	14
6	14
7	15
8	15

From the foregoing results, it can be seen that a strong beautifying and whitening effect was shown when dihydromyricetin was compounded in amounts of greater than 0.001 weight % and that this effect was marked at greater than 0.005 weight %. Next, examples of this invention will be presented. However, this invention is not limited by them. The compounding quantities are weight %.

Example 1 -- Toilet Water

(1) Dihydromyricetin	0.5
(2) Glycerol	4.0
(3) 1,3-butylene glycol	3.0
(4) Ethyl alcohol	7.0
(5) Polyoxyethylene (20) lauryl ether	0.5
(6) Methyl paraoxybenzoate	0.1
(7) Citric acid	0.01
(8) Sodium citrate	0.1
(9) Perfumes	suitable quantity

(10) Purified water added to make 100 per cent

Components (1) to (4), component (6) and component (9) were mixed and dissolved. Separately, components (5), (7), (8) and (10) were mixed and dissolved. Next, the two solutions were mixed. They were then filtered with Tetron cloth (300 mesh) with the product being obtained.

Example 2 -- Cream

(1) Dihydromyricetin	0.1
(2) Squalane	11.5
(3) Cetyl alcohol	2.5
(4) Polyoxyethylene (20) sorbitan monosulfate	1.0
(5) Polyoxyethylene (20) cetyl ether	2.5
(6) 1,3-butylene glycol	4.0
(7) Propylene glycol	3.5
(8) Titanium dioxide	7.0
(9) Red oxide of iron	0.5
(10)Yellow iron oxide	0.2
(11) Black iron oxide	0.1
(12) Methyl paraoxybenzoate	0.3
(13) Perfumes	suitable quantity

(14) Purified water added to make 100 per cent

Components (1) to (5) were heated, dissolved and mixed and the mixture was maintained at 70°C, with an oleaginous phase being prepared. Components (6) to (12) were dispersed uniformly in component (14) and maintained at 75°C, with an aqueous phase being prepared. The aqueous phase was added to the oleaginous phase and emulsification and dispersion were effected.

Component (13) was added and the mixture was cooled to 30°C as it was being stirred, with the product being obtained.

Example 3 -- Cream

(1) Dihydromyricetin	0.05
(2) Squalane	5.5
(3) Olive oil	3.0
(4) Stearic acid	2.0
(5) Beeswax	2.0
(6) Octyldocecyl myristate	3.5
(7) Polyoxyethylene (20) cetyl ether	3.0
(8) Behenyl alcohol	1.5
(9) Glycerol monostearate	2.5
(10) 1,3-butylene glycol	8.5
(11) Ethyl paraoxybenzoate	0.2
(12) Perfumes	suitable quantity

(13) Purified water added to make 100 per cent

Components (1) to (9) were added, dissolved and mixed and the mixture was maintained at 70°C, with an oleaginous phase being prepared. Components (10) and (11) were mixed by heating and were dissolved in component (13) and the mixture was maintained at 75°C, with an aqueous phase being prepared. The aqueous phase was added to the oleaginous phase, component (12) was and emulsification was performed uniformly, after which the emulsion was cooled to 30°C as it was being stirred, with the product being obtained.

Example 4 - Emulsion

(1) Dihydromyricetin	2.0
(2) Squalane	5.0
(3) Olive oil	5.0
(4) Hohoba [phonetic] oil	5.0
(5) Cetyl alcohol	1.5
(6) Glycerol monostearate	2.0
(7) Polyoxyethylene (20) cetyl ether	3.0
(8) Polyoxyethylene (20) sorbitan monostearate	2.0
(9) Glycerol	7.0
(10) Perfumes	Suitable quantity
(11) Methyl paraoxybenzoate	0.3

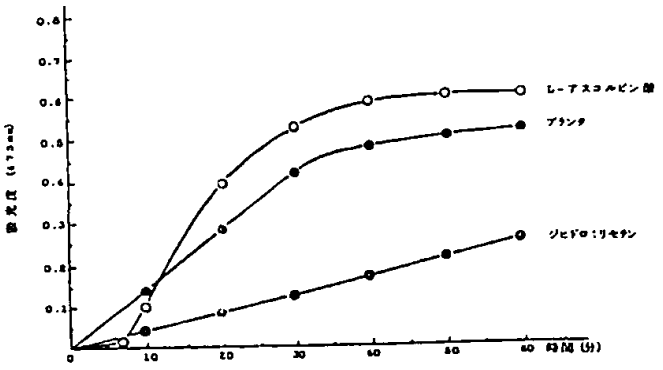
(12) Purified water added to make 100 per cent

Components (1) to (8) were heated, dissolved and mixed and the mixture was maintained at 70°C, with an oleaginous phase being prepared. Components (9) and (11) were added to and dissolved and mixed in (12) and the mixture was maintained at 75°C, with an aqueous phase being prepared. The aqueous phase was added to the oleaginous phase, component (10) was added and emulsification was performed uniformly, after which the emulsion was cooled to 30°C while being stirred, with the product being obtained.

4. Brief Explanation of the Figure

The graph is a figure that shows the relationship between degree of coloration and time for the purpose of indicating the tyrosinase activity inhibiting power of the aqueous solutions in Example 1.

Applicant: Nonogawa Shoji Company, Ltd.



[vertical axis]: Absorbance (475 mm)
[horizontal axis]: Time (minutes)
[Curves inside graph, top to bottom]
L-ascorbic acid
Blank
Dihydromyricetin

⑲日本国特許庁（JP）　⑪特許出願公開

⑫公開特許公報（A）　昭63－316711

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⑤4発明の名称　美白化粧料

⑳特　願　昭62－151019

㉒出　願　昭62(1987)6月17日

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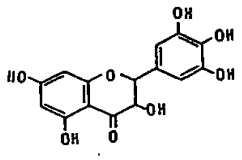
明　細　書

1．発明の名称

美白化粧料

2．特許請求の範囲

（1）下記の一般式で表されるジヒドロミリセチンを有効成分とする美白化粧料。



（2）ジヒドロミリセチンが、0.001～5.0重量％含有せられてなる特許請求の範囲第1項記載の美白化粧料。

3．発明の詳細な説明

〔産業上の利用分野〕

本発明は新規な皮膚美白化粧料に関する。さらに詳しくは、ジヒドロミリセチン（Dihydromyricetin：$C_{15}H_{12}O_8$：アンペロプシン：または3,5,7,3′4′,5′－ヘキサヒドロキシフラバノンとも言うが、以下ジヒドロミリセチンと称す）を有効成分として含有せしめた美白効果の大なる色白化粧料に関する。

〔従来の技術〕

皮膚のしみ、色黒、そばかすなどの発生機序については不明な点もあるが、一般には、ホルモンの異常や日光からの紫外線の刺激が原因となってメラニン色素が形成され、これが皮膚内に異常沈着するものと考えられる。このようなしみやあざなどの治療法にはメラニンの生成を抑制する物質、例えば、L-アスコルビン酸を大量に経口投与する方法、グルタチオン等を注射する方法、あるいはL-アスコルビン酸、システインなどを軟膏、クリーム、ローションなどの形態にして局所に塗布する方法などがとられている。また、欧米ではハイドロキノン製剤がしみ、そばかすなどの治療薬として用いられている。

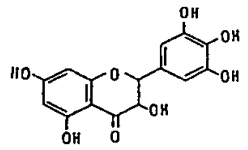
〔発明が解決しようとする問題点〕

L-アスコルビン酸類はその経時的な安定性の面で問題があり、特に水分を含む系では不安定で、変色、変臭の原因となる。一方、ハイドロキノンは効果は大きいが感作性があるため一般には使用が制限されており、また、空気酸化されやすいため安定性の面においても問題がある。グルタチオン、システインなどのチオール系化合物は、異臭が強い上、酸化されやすく効果も緩慢である。また、2-メルカプトエチルアミル塩酸塩およびN-（2-メルカプトエチル）ジメチルアミン塩酸塩等は、黒色モルモットを脱色することが知られているが、これらの化合物は不安定なうえ刺激性が強く、脱色後に白斑が生じやすいので一般には使用されていない。

〔問題を解決するための手段〕

本発明者等は、このような事情に鑑み、鋭意研究を重ねた結果、フラボノイドのジヒドロミリセチンが、良好な皮膚美白効果を発揮することを認め本発明を完成するに至った。

すなわち、本発明は、下記一般式で表されるジヒドロミリセチンを有効成分とする美白化粧料である。



本発明のジヒドロミリセチンは合成品でも天然物から抽出した物でも良い。また天然品の場合は、純品でなくジヒドロミリセチンを含む混合物であっても良い。

天然物からの抽出方法としては、例えば以下のような効率的な分画、抽出方法を用いることができる。水野ら（水野瑞夫、田中稔幸、飯沼宗和、木村有香、大橋広好、境秀紀；日本生薬学会第32回年会講演予稿集、P 51.1985 年岡山）の方法に従ってオノエヤナギ（Salix sachalinensis Fr. Schm.）、ケショウヤナギ（Chosenia bracteosa Nakai)等のヤナギ科の植物の成葉をメタノール抽出し、濃縮後、エーテル、ついで酢酸エチルを用いて分配し、得られた酢酸エチル層をさらにシリカゲルカラムクロマトグラフィーにより分画、精製を行って得ることができる。本発明の実施に当たってジヒドロミリセチンの配合量は、化粧料全量中0.001～5重量%、好ましくは 0.005 ～3重量%である。 0.001重量%以下であると本発明で言う効果が充分に発揮されず好ましくない。また、5重量%以上の配合も可能であるが、効果の顕著な増強も認められず、また経済的にも好ましくない。

本発明の化粧料は前記の必須成分に加えて必要に応じて本発明の効果を損なわない範囲内で、化粧品一般に用いられる各種成分すなわち油脂類、ロウ類、炭化水素類、脂肪酸類、アルコール類、合成エステル類、界面活性剤、保湿剤、増粘剤、無機物、香料、薬剤、水等を配合することができる。

〔発明の効果〕

本発明の美白化粧料は、強いチロジナーゼ活性阻害効果を有する。 また、本出願人が特許出願（特願昭 62-041173）で開示したように、安全性に富み、水およびアルコールに可溶性であるので、化粧料としての利用が容易である。

つぎに実験例、をあげて本発明の効果をさらに詳しく説明する。

〔実験例-1〕

ジヒドロミリセチンを水に溶解し、濃度1.0 mMのジヒドロミリセチン水溶液を得た。この水溶液のチロジナーゼ活性阻害力を調べた結果を次に説明する。

試験管にL-チロジン溶液（0.3 mg/ml）を1 ml、マックルベイン氏の緩衝液（pH 6.8）を 1 ml、およびジヒドロミリセチン水溶液0.9 mlを加えて37℃の恒温水槽中で、10分間インキュベートした後、これにチロジナーゼ溶液（1 mg/ml）を、0.1 ml加え、撹はんし、直ちに分光光度計にて475 nmにおける吸光度を経時的に測定した。

一方、ブランクテストとして前記水溶液の代わりに水を用いて同様の吸光度測定を行った。

つぎに、比較例としてアスコルビン酸を用いて実

験例と同様にして水溶液を調製し、そのチロジナーゼ活性阻害力を調べた。

これら実験例、及び比較例における各試験結果を添付図面（グラフ）に示す。このグラフから実験例-1で得た水溶液はアスコルビン酸からなる水溶液に比べて顕著なチロジナーゼ活性阻害力を有していることがわかる。さらに、アスコルビン酸のチロジナーゼ活性阻害力が経時的に低下するのに比べ、ジヒドロミリセチンからなる水溶液は経時的に安定で、一定の阻害力を有することも本発明の特徴である。

つぎに、本発明による美白化粧料の美白効果を実験例-2によって説明する。

〔実験例-2〕

ジヒドロミリセチンの配合量を変化させ美白効果を検討した。実施例-1の化粧水のジヒドロミリセチンの配合量を変化させ第1表に示す試料（No1～No8）を調整した。

第1表

試料No	ジヒドロミリセチン配合量（重量%）
1	0
2	0.001
3	0.005
4	0.01
5	0.1
6	1.0
7	3.0
8	5.0

色黒、しみ、そばかす等に悩む被験者40名をパネラーとし、各試料につき5名ずつテストを行った。1カ月間毎日顔面に化粧水を塗布させ、使用後の淡色化効果を下記の判定基準にもとずいて判定した。判定結果を第2表に示す。

（判定基準）

3点：色素沈着が目立たなくなった。

2点：色素沈着がかなり薄くなった。

1点：色素沈着がやや薄くなった。

0点：色素沈着に変化がなかった。

第2表

試料No	被験者5名の合計点
1	1
2	8
3	12
4	14
5	14
6	14
7	15
8	15

上記の結果よりジヒドロミリセチンを0.001重量%以上配合した場合強い美白効果を示し、0.005重量%以上では、その効果は顕著であることがわかる。次に本発明の実施例を上げるが、本発明はこれにより限定されるものではない。配合量は重量%である。

実施例－1　化粧水

①ジヒドロミリセチン	0.5
②グリセリン	4.0
③1,3-ブチレングリコール	3.0
④エチルアルコール	7.0
⑤ポリオキシエチレン（20）ラウリルエーテル	0.5
⑥パラオキシ安息香酸メチル	0.1
⑦クエン酸	0.01
⑧クエン酸ナトリウム	0.1
⑨香料	適量

⑩精製水を加えて100とする。

成分①～④、成分⑥、および⑨を混合して溶解する。別に成分⑤、⑦、⑧、および⑩を混合して溶解する。ついで両者を混合し、テトロン製布（300メッシュ）により濾過し、製品とする。

実施例－2　クリーム

①ジヒドロミリセチン	0.1
②スクワラン	11.5